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JACOBSON HOLMAN PLLC
400 SEVENTH STREET N.W.
SUITE 600
WASHINGTON, DC 20004

EXAMINER	
FOSTER, CHRISTINE E	
ART UNIT	PAPER NUMBER
1641	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/05/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/507,479	REGINSTER ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 January 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) *10-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9* is/are rejected.

7)☒ Claim(s) *2,3,5 and 9* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 September 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/13/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of Group I, claims 1-10 in the reply filed on 11/6/06 and in the subsequent reply of 1/5/07 is acknowledged. The election with traverse of HRGYPGLDG (claim 9) as the species of amino acid sequence in the reply filed of 1/5/07 is further acknowledged.

2. The traversal relates to the election of species requirement and is on the ground(s) that the species are related to a single general inventive concept in that they both include nitrated tyrosine (see reply, p. 2). Applicant further argues that Beckman does not teach or suggest the present application as claimed, in that Beckman does not teach measuring a specific sequence that undergoes nitration during a pathological process, and further that the antiserum described in the present application does not recognize free nitrotyrosine as does Beckman's.

3. This is not found persuasive because the record as set forth in the previous Office action shows that the feature of "amino acid sequences containing nitrated tyrosine" is taught in the prior art (see the previous Office action at p. 3 and p. 5-6). Such a feature is also taught in Paik et al. (see the previous Office action at p. 3). As such, the species of amino acid sequences are not regarded as being of a similar nature because the shared common structure (nitrotyrosine) is not a contribution over the prior art; therefore the technical feature of nitrotyrosine cannot be considered a *special* technical feature linking the species, as it does not represent a contribution over the prior art.

4. Applicant's arguments that the antiserum of Beckman recognizes free nitrotyrosine, while the antiserum of the present application does not, are not persuasive because Applicant is referring to limitations that do not appear in the claims. Specifically, Applicant appears to argue

that the antiserum of the invention recognizes nitrotyrosine in a *context-dependent* manner, while that of Beckman recognizes nitrotyrosine in a *context-independent* manner. However, the claims encompass both context-dependent and context-independent recognition of nitrotyrosine. For example, claim 4 specifically recites an immunological binding partner that recognizes a nitrated form of an aromatic amino acid residue (e.g., nitrotyrosine) in a *context-independent* manner.

In addition, this argument is also unpersuasive since the election of species requirement pertains to *amino acid sequences* and not to *antisera* that recognize such sequences; for example, claims 9-10 do not recite an antiserum. As noted above, since amino acid sequences containing nitrotyrosine were known in the prior art as taught by Beckman and Paik et al., the species of amino acid sequences lack unity of invention. Even if antiserum were at issue, since the antiserum of Beckman recognizes nitrotyrosine *per se*, it also recognizes nitrotyrosine residues in the context of a larger amino acid sequence (see for example the abstract).

The requirement is still deemed proper and is therefore made FINAL.

5. Claims 10-26 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to nonelected species (claim 10) and inventions (claims 11-26), there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 1/5/07.

Oath/Declaration

6. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

> The oath or declaration is defective because:
> It does not identify the city and either state or foreign country of residence of each inventor. The residence information may be provided on either an application data sheet or supplemental oath or declaration.

Specifically, the residence address for Inventor Stephan Christgau is not listed on the oath nor on an application data sheet.

Priority

7. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Specifically, it is noted that the Oath refers to **U.S. Provisional Application 60/363,925**, filed on 03/13/2002. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

8. If applicant desires to claim the benefit of a prior-filed application under 35 U.S.C. 119(e), a specific reference to the prior-filed application in compliance with 37 CFR 1.78(a) must be included in the first sentence(s) of the specification following the title or in an application data sheet. For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of the applications.

If the instant application is a utility or plant application filed under 35 U.S.C. 111(a) on or after November 29, 2000, the specific reference must be submitted during the pendency of the application and within the later of four months from the actual filing date of the application or sixteen months from the filing date of the prior application. If the application is a utility or plant application which entered the national stage from an international application filed on or after November 29, 2000, after compliance with 35 U.S.C. 371, the specific reference must be

submitted during the pendency of the application and within the later of four months from the date on which the national stage commenced under 35 U.S.C. 371(b) or (f) or sixteen months from the filing date of the prior application. See 37 CFR 1.78(a)(2)(ii) and (a)(5)(ii). This time period is not extendable and a failure to submit the reference required by 35 U.S.C. 119(e) and/or 120, where applicable, within this time period is considered a waiver of any benefit of such prior application(s) under 35 U.S.C. 119(e), 120, 121 and 365(c). A benefit claim filed after the required time period may be accepted if it is accompanied by a grantable petition to accept an unintentionally delayed benefit claim under 35 U.S.C. 119(e), 120, 121 and 365(c). The petition must be accompanied by (1) the reference required by 35 U.S.C. 120 or 119(e) and 37 CFR 1.78(a)(2) or (a)(5) to the prior application (unless previously submitted), (2) a surcharge under 37 CFR 1.17(t), and (3) a statement that the entire delay between the date the claim was due under 37 CFR 1.78(a)(2) or (a)(5) and the date the claim was filed was unintentional. The Director may require additional information where there is a question whether the delay was unintentional. The petition should be addressed to: Mail Stop Petition, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

If the reference to the prior application was previously submitted within the time period set forth in 37 CFR 1.78(a), but not in the first sentence(s) of the specification or an application data sheet (ADS) as required by 37 CFR 1.78(a) (e.g., if the reference was submitted in an oath or declaration or the application transmittal letter), and the information concerning the benefit claim was recognized by the Office as shown by its inclusion on the first filing receipt, the petition under 37 CFR 1.78(a) and the surcharge under 37 CFR 1.17(t) are not required.

Applicant is still required to submit the reference in compliance with 37 CFR 1.78(a) by filing an amendment to the first sentence(s) of the specification or an ADS. See MPEP § 201.11.

Information Disclosure Statement

9. Applicant's Information Disclosure Statement filed 1/13/2005 has been received and entered into the application. The references therein have been considered by the examiner as indicated on the attached form PTO-1449.

10. Citation AE (WO 98/29452) was considered by the examiner only to the extent of the abstract, because only the abstract is in English.

11. Applicant is reminded that the listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Specification

12. The disclosure is objected to because of the following informalities:

13. Required subject headings, such as the "BACKGROUND OF THE INVENTION", "BRIEF SUMMARY OF THE INVENTION", and "BRIEF DESCRIPTION OF THE DRAWINGS", are absent.

The following guidelines illustrate the preferred layout for the specification of a utility application. These guidelines are suggested for the applicant's use.

Arrangement of the Specification

As provided in 37 CFR 1.77(b), the specification of a utility application should include the following sections in order. Each of the lettered items should appear in upper case, without underlining or bold type, as a section heading. If no text follows the section heading, the phrase "Not Applicable" should follow the section heading:

(a) TITLE OF THE INVENTION.
(b) CROSS-REFERENCE TO RELATED APPLICATIONS.
(c) STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT.
(d) THE NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT.
(e) INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A COMPACT DISC.
(f) BACKGROUND OF THE INVENTION.
(1) Field of the Invention.
(2) Description of Related Art including information disclosed under 37 CFR 1.97 and 1.98.
(g) BRIEF SUMMARY OF THE INVENTION.
(h) BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S).
(i) DETAILED DESCRIPTION OF THE INVENTION.
(j) CLAIM OR CLAIMS (commencing on a separate sheet).
(k) ABSTRACT OF THE DISCLOSURE (commencing on a separate sheet).
(l) SEQUENCE LISTING (See MPEP § 2424 and 37 CFR 1.821-1.825. A "Sequence Listing" is required on paper if the application discloses a nucleotide or amino acid sequence as defined in 37 CFR 1.821(a) and if the required "Sequence Listing" is not submitted as an electronic document on compact disc).

Appropriate correction is required.

Claim Objections

14. Claims 2-3, 5, and 9 are objected to because of the following informalities:

15. Claims 2-3 refer to "said aromatic amino acid residue"; however, claim 1 refers to "one or more aromatic amino acid residues".

16. Claim 3 refers to "a specific protein" in line 3, which apparently refers back to the "specific protein" recited earlier in claim 1. For clarity, it is suggested that claim 3 refer to "**said** specific protein" or "**the** specific protein".

17. Claim 5 refers to the specific protein "of which the detected nitrated amino acid sequence is **detected**", which should apparently read –of which the detected nitrated amino acid sequence is **characteristic**--.

18. Claim 9 recites the sequence HRGYPGLDG and is accompanied by the sequence identifier "SEQ ID NO: 6". However, the recited sequence does not match that given for SEQ ID NO:6 in the sequence listing filed on 1/5/2007. Clarification and/or correction are requested.

19. Claim 9 is objected to because the sentence structure is ambiguous and may cause confusion. Specifically, it is not clear whether the clause "or is comprised within said sequence and includes said nitrated tyrosine" is modifying the subject "the amino acid sequence" or the subject "the amino acid residue Y". For the purposes of examination the claim was assumed to recite that "the amino acid sequence which is detected" either (1) comprises the sequence HRGYPGLDG (SEQ ID NO:6) in which the amino acid residue Y is nitrated tyrosine; or (2) is comprised within said sequence and includes said nitrated tyrosine.

Claim Rejections - 35 USC § 112

20. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

21. Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to methods of detecting an "**amino acid sequence that is characteristic of a specific protein and which contains one or more aromatic amino acid residues in nitrated form**". The methods may be conducted as an immunoassay (see claim 2) using an "**immunological binding partner**" that is immunoreactive with the nitrated form of the aromatic amino acid residue (see claims 2-4). A second "**immunological binding partner**" that is specific for an amino acid sequence that is characteristic of the specific protein may also be employed (claim 5). The claims are therefore drawn to a genus of methods of detecting a genus of protein sequences that include a nitrated amino acid residue.

The specification discloses with particularity only two specific amino acid sequences that contain aromatic amino acid residues in nitrated form:

HRGYPGLDG (SEQ ID NO:6)
Characteristic of collagen type II protein (see page 7, lines 23-30; page 12, lines 24-30; and Example 1).

LGYMRA (SEQ ID NO:7)
Also characteristic of collagen type II (see page 12, lines 24-30).

No other amino acid sequences that are "characteristic of" specific proteins are disclosed with any particularity. The disclosure of the two species of amino acid sequences does not fully support the instant claims drawn to the genus of all "amino acid sequences that are characteristic

of a specific protein and which contain one or more aromatic amino acid sequences in nitrated form".

The size and substantial variability of this claimed genus of amino acid sequences to be detected is clearly at issue. Such sequences could be presumably of any length; however, taking for example the preferred embodiment in which the sequences are detected by antibodies, it is known that the smallest peptide that will consistently elicit antibodies are 6 residues in length (see Harlow, E. and Lane, D., Antibodies: A Laboratory Manual (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, at page 76). The genus of amino acid sequences is also not limited to nitrotyrosine. However, taking just the example of nitrotyrosine in the context of a 6-residue amino acid sequence, this would mean a minimum genus size on the order 20^5 different sequences. The disclosure of only two specific sequences (SEQ ID NO:6-7) fails to support claims drawn to a genus of detecting more than two million sequences, given the substantial variability among members of the genus.

Although the members of the genus share in common a nitrated aromatic amino acid residue, such a partial structural characteristic fails to convey evidence of possession of methods of detecting all amino acid sequences having such a residue, for the following reasons.

The specification discloses that the nitrated amino acid sequences may be detected by immunoassay, in which an antibody is employed which is reactive with the nitrated amino acid residue. It is noted the claims currently encompass both "context-independent" and "context-dependent" antibodies. "Context-independent" antibodies are those that specifically bind to (for example) nitrotyrosine *per se*, regardless of the identity of the surrounding amino acids. By contrast, "context-dependent" antibodies would only recognize nitrotyrosine when it is in the

context of other specific amino acids. See the specification, for example at page 4, line 25 to page 5, line 8. In other words, the epitope recognized by "context-independent" antibodies is nitrotyrosine itself (or another nitrated amino acid residue), while the epitope recognized by "context-dependent" antibodies is defined not only by the nitrotyrosine residue but also includes surrounding amino acids.

The courts have stated that "as long as an applicant has disclosed a "fully characterized antigen," either by its structure, formula, chemical name, or physical properties, or by depositing the protein in a public depository, the applicant can then claim an antibody by its binding affinity to that described antigen." *Noelle v. Lederman*, 355 F.3d at 1349 (Fed. Cir. 2004, emphasis in the original). Although *Noelle* relates to antibodies *per se* and not to detecting methods using such antibodies, the holdings of those cases are also applicable to claims such as those at issue here. A disclosure that does not adequately describe a product itself logically cannot adequately describe a method of using that product.

Applicant has failed to provide written description for the currently claimed genus of detection methods using either "context-dependent" or "context-independent" antibodies.

In the case of "context-independent" detection of nitrotyrosine-containing sequences using antibodies that recognize nitrotyrosine *per se,* one skilled in the art would accept that an adequate description of the specific antigen epitope (nitrotyrosine) would put an inventor in possession of antibodies that bind to nitrotyrosine in a context-independent manner, given that production of antibodies against a well-characterized antigen was conventional at the time of filing.

However, Applicant is claiming a method of detecting "an amino acid sequence which is **characteristic of a specific protein**" (emphasis added). Applicant has not described how context-independent antibodies that recognize nitrotyrosine in any context may be used to recognize such specific amino acid sequences, since by definition, antibodies that are context-independent would bind to all nitrotyrosine-containing sequences, and thus would not specifically detect certain "characteristic" sequences.

It is noted that the method of claim 4 uses a first context-independent antibody (e.g., anti-nitrotyrosine antibody) followed by a second antibody that is specific for "an amino acid sequence which is characteristic of" the specific protein to be detected. Even if the specification were to demonstrate possession of such a two-antibody method, the claims are not so limited, such that the specification's disclosure of this two-antibody method fails to convey evidence of possession of all assay methods that employ context-independent antibodies.

However, even in the case of claim 4, Applicant has failed to provide adequate written description since although context-independent anti-nitrotyrosine are adequately described by reference to the antigen epitope (nitrotyrosine), the "amino acid sequences that are characteristic of a specific protein" that are bound by the second immunological binding partner lack written description. Such amino acid sequences presumably refer to other, non-nitrated portions of the specific protein, yet Applicant has described **no** such amino acid sequences with any particularity. Applicant has also not described any second antibodies that recognize such sequences. Neither the recited "amino acid sequences" nor the "second immunological binding partner" is adequately described in the specification. Applicant is attempting to describe an unknown by reference to another unknown.

Turning now to methods that use "context-dependent" antibodies, Applicant has not adequately described the genus of sequences to be detected since Applicant has described neither specific antibodies that would recognize such sequences, nor any partial structure that would be shared by such sequences—i.e., the antibody epitope (with the two limited exceptions of SEQ ID NO:6-7). In order to convey evidence of possession of methods to detect *specific* amino acid sequences that are characteristic of a *specific* protein, Applicant would have to describe either the "immunological binding partner" used to detect such sequences, or alternatively, the fully characterized structure of the antigen. The specification does not describe the complete structure of any antibody that binds to an amino acid sequence "which is characteristic of a specific protein and which contains one or more aromatic amino acid residues in nitrated form". The disclosure of only two species (SEQ ID NO:6-7) as epitopes that may be recognized by an antibody fails to convey evidence of possession of the entire genus because by definition, context-dependent antibodies raised against one specific sequence would **not** recognize other nitrated sequences.

Although Applicant has named specific proteins in claim 7, it is noted that the claims do not relate to detection of these proteins *per se*, but rather to the detection of amino acid sequences that are "characteristic" of such proteins, which would include not only detection of these proteins but of various fragments thereof (see for example the specification at page 5, lines 6-8, and at page 6, lines 5-14). Further, since Applicant has not provided a limiting definition for what would represent an amino acid sequence that is "**characteristic of**" a specific protein (see rejection under 112, 2nd paragraph below), the claims would also reasonably encompass amino acid sequences that are homologous to specific proteins, related in terms of three-dimensional

structure, modified forms, etc., such that the claims are not limited only to fragments of specific proteins. However, Applicant has not adequately described these various scenarios that would be encompassed by the claims.

The reference to known proteins in claim 7 fails to support the claimed methods of detecting sequences that are "characteristic of" these proteins, since the claims are not limited to detection of these proteins. The structure of the sequences that are actually detected in the method is not adequately described in the specification. One cannot envisage possession since the structure of the antigen is not fully characterized. Although one skilled in the art may envisage possession of antibodies against collagen type I based on the disclosure of this known protein antigen, such a disclosure fails to convey evidence of possession of amino acid sequences "characteristic of" collagen type I or of antibodies that bind such sequences. Consequently, one also cannot envisage possession of methods of detecting all such sequences.

In particular, with regard to a preferred embodiment in which fragments of specific proteins are to be detected, although such fragments all include a nitrated amino acid, Applicant has not identified any partial structure or other relevant identifying characteristics shared by the fragments to be detected. As noted above, the nitrated amino acid alone is not enough to define a context-dependent antibody epitope. Thus, the reference to known proteins is not sufficient to adequately describe methods of detecting all fragments thereof, since the structure of the fragments that are actually detected is unknown.

As a result, even in the case of claim 7 where specific proteins are named, since the claims are not limited to detection of these proteins, neither the antigen (amino acid sequence) nor the antibody that would be used to detect the antigen are described

Thus, although the protein sequences to be detected all include a nitrated amino acid residue, one skilled in the art cannot envisage detection of the genus of all nitrated protein sequences since there is no correlation between the presence of the nitrated amino acid residue and function (ability to bind to an antibody) that is shared among the members of the genus. The presence of a nitrated amino acid residue is not enough to adequately define the genus of sequences to be detected, since it is not enough to define a context-dependent antibody epitope. As such, with the exception of the two disclosed species, Applicant has not adequately described either the antibody itself or the epitope to be recognized.

In addition, the claims would encompass detection of nitrated amino acid sequences where the epitope is either linear or conformational. In other words, the other amino acids that participate in antibody binding could be close together either in the primary amino acid sequence (as part of a linear epitope), or alternatively could be residues that are far apart in the protein's primary amino acid sequence but which come together in the three-dimensional folded protein. See Harlow & Lane, pages 23-25, especially page 25, the first paragraph. However, Applicant has also not described any methods of making context-dependent antibodies that recognize conformational epitopes (i.e., epitopes formed by residues that are brought together in the folded structure of the protein), since Applicant has not described the three-dimensional structures of any specific proteins to be detected. As such, one skilled in the art cannot envisage possession of methods of detecting nitrated amino acid residues in the context of a conformational epitope since there is no description for any protein of the amino acid residues that are close together in space to a nitrated amino acid residue.

With respect to claim 9, which recites the elected species of HRGYPGLDG (SEQ ID NO:6, where the tyrosine is nitrotyrosine), the claim lacks written description for the following reasons. The claim is therefore drawn to a genus of methods of detecting all amino acid sequences that comprise this sequence. This genus would include (for example) detection of the full-length, native collagen type II protein (in nitrated form), since SEQ ID NO:6 is a portion of collagen type II derived form the triple helical region (see page 20).

However, the specification makes clear that antibodies raised against HRGY(NO_2)PGLDG (SEQ ID NO:6) did not bind to native, full-length type II collagen (Example 2). See also page 30, lines 5-6, where it is disclosed that "[t]he nitrosylated sequence is recognized *only in the form of free fragments*" (emphasis added). Consequently, one skilled in the art cannot envisage possession of methods of detecting all sequences comprising HRGY(NO_2)PGLDG (SEQ ID NO:6) since Applicant has not described any methods of detecting the full-length protein comprising that sequence.

The postfiling literature provides further evidence that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of the native protein, but rather is only detectable in linear, unwound fragments of collagen type II that no longer retain the triple helix structure of wound collagen.

For example, Manicourt et al. ("Products of Cartilage Metabolism", In: Dynamics of Bone and Cartilage Metabolism (2006), Eds. Markus J. Seibel et al., Academic Press, Chapter 25, pages 421-449) teach that native, fully wound collagen is cleaved by proteases to yield denatured collagen, which exposes neoepitopes that are normally hidden and therefore not recognized in the native triple helix (see page 425, right column, the last paragraph). Such

neoepitopes include the Coll2-1-NO2 peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) that is disclosed in the instant specification (see Table 1 and page 428, left column). Manicourt et al. therefore teach that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of native, wound collagen type II, consistent with the data presented in the specification.

Applicant's postfiling work also identifies the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence as a neoepitope that is not present in either native or in heat-denatured type II collagen, but only in the context of linear fragments. See Deberg et al. ("New serum biochemical markers (Coll 2-1, and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis" *OsteoArthritis and Cartilage* (2005) 13, 258-265, especially p. 260, right column, "Antiserum specificity"; and Figure 1). For example, Deberg et al. teach that an antisera (D3) specific for the HRGYPGLDG epitope (termed "Coll 2-1") did not bind to heat-denatured type II collagen, "suggesting that D3 was *specific for the linear form* of Coll 2-1" (page 260, right column, the second to last paragraph, emphasis added).

Therefore, in describing only antibodies that recognize the neoepitope sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), Applicant has not described how to detect all sequences that include this sequence, since only unwound, linear fragments present the neoepitope sequence. The specification does not describe how to detect <u>all sequences</u> that comprise this sequence, or even how to detect <u>all fragments</u> that comprise this sequence, since only *unwound, linear fragments of collagen type II* would be capable of being detected by the antibodies disclosed in the specification.

Finally, it is noted that the claims are not restricted to *antibodies*, but rather refer to "**immunological binding partners**" (see claims 2-4). Although Applicant has described art-recognized methods of making antibodies (see page 16, line 24 to page 17, line 3), the specification does not provide adequate written description for the genus of "**immunological binding partners**" because the specification does not disclose any partial structure, physical and/or chemical properties, or other relevant identifying characteristics shared by members of the genus. The genus of "immunological binding partners" is described only by reference to a functional characteristic (ability to immunoreact with an amino acid sequence or nitrated amino acid residue); however, there is no disclosed correlation between structure and function. Applicant has also not described any methods of making immunological binding partners other than antibodies.

In summary, Applicant has described only two amino acid sequences with any particularity, yet the claims are drawn to methods of detecting *any* amino acid sequence that contains a nitrated aromatic amino acid. The specification does not describe a representative number of amino acid sequences to be detected, and therefore fails to convey evidence of possession in light of the large size and substantial variability of the genus being claimed. Specifically, since the partial structure of a nitrated amino acid residue is not enough to define a context-dependent antibody epitope, Applicant has not described how to detect all specific nitrated amino acid-containing sequences. Accordingly, in the absence of sufficient recitation of distinguishing identifying characteristics, the specification does not provide adequate written description of the claimed genus.

22. Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for detecting the sequence consisting of HRGY(NO_2)PGLDG (SEQ ID NO:6) using context-dependent antibodies raised against SEQ ID NO:6 that do not bind to HRGYPGLDG (SEQ ID NO:1), does not reasonably provide enablement for methods of detecting **all** amino acid sequences that comprise the sequence HRGY(NO_2)PGLDG (SEQ ID NO:6) by any means. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The nature of the invention relates to the production of antisera against peptides consisting of the sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), where Y(NO_2) is nitrotyrosine, and the use of such antisera in detection methods involving serum. See especially page 7, lines 23-27 and Examples 1, 5, and 6. The antisera were successfully used in methods of detecting the eliciting peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) (see Example 2).

The antisera had strong affinity to the nitrated sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), but only weak affinity to the non-nitrated sequence HRGYPGLDG (SEQ ID NO:1) (Example 1). This means that the epitope recognized by the antisera included the nitrotyrosine residue, such that the antisera discriminate the nitrated from the non-nitrated sequence.

The antisera produced against the peptides were seen to have no binding affinity to free L-nitrotyrosine (see Example 2, especially at page 23, lines 5-6), such that the antisera are *context-dependent*, i.e. they do not bind to free nitrotyrosine, but rather, only recognize the nitrotyrosine residue that appears in the context of SEQ ID NO:6. See the written description rejection above for further discussion of context-dependent vs. context-independent antibodies.

By contrast, the claims are broadly drawn to methods of detecting **all** amino acid sequences that are "characteristic of" (any) specific protein and which contain one or more amino acid residues in nitrated form. This would include not only nitrotyrosine, but also various nitrated forms of tyrosine, phenylalanine, tryptophan (the aromatic amino acid residues).

The specification discloses that HRGY(NO_2)PGLDG (SEQ ID NO:6) is an amino acid sequence that is "characteristic of" collagen type II, in that this sequence corresponds to a portion of the triple helical region of the protein (see page 12, lines 26-30). Since claim 9 recites that the amino acid sequence to be detected "comprises" the sequence HRGYPGLDG (where the tyrosine is nitrated) the claims clearly encompass detection of the full-length, native collagen type II protein, since the full-length sequence comprises that sequence.

However, the specification makes clear that antibodies raised against HRGY(NO_2)PGLDG (SEQ ID NO:6) did not bind to native, full-length type II collagen (Example 2). See also page 30, lines 5-6, where it is disclosed that "[t]he nitrosylated sequence is recognized only in the form of free fragments". Thus, the specification provides no direction or guidance and no working examples with regard to how to detect native, full-length type II collagen comprising the recited sequence.

The postfiling literature provides further evidence that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of the native protein, but rather is only detectable in linear, unwound fragments of collagen type II that no longer retain the triple helix structure of wound collagen.

For example, as discussed above, Manicourt et al. ("Products of Cartilage Metabolism", In: Dynamics of Bone and Cartilage Metabolism (2006), Eds. Markus J. Seibel et al., Academic

Press, Chapter 25, pages 421-449) teach that native, fully wound collagen is cleaved by proteases to yield denatured collagen, which exposes neoepitopes that are normally hidden and therefore not recognized in the native triple helix (see page 425, right column, the last paragraph). Such neoepitopes include the Coll2-1-NO2 peptide HRGY(NO_2)PGLDG (SEQ ID NO:6) that is disclosed in the instant specification (see Table 1 and page 428, left column). Manicourt et al. therefore teach that the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence is not accessible to antibody binding in the context of native, wound collagen type II, consistent with the data presented in the specification.

Applicant's postfiling work also identifies the HRGY(NO_2)PGLDG (SEQ ID NO:6) sequence as a neoepitope that is not present in either native or heat-denatured type II collagen, but only in the context of linear fragments. See Deberg et al. ("New serum biochemical markers (Coll 2-1, and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis" *OsteoArthritis and Cartilage* (2005) 13, 258-265, especially p. 260, right column, "Antiserum specificity"; and Figure 1). For example, Deberg et al. teach that an antisera (D3) specific for the HRGYPGLDG epitope ("Coll 2-1") did not bind to native or heat-denatured type II collagen, "suggesting that D3 was *specific for the linear form* of Coll 2-1" (page 260, right column, the second to last paragraph, emphasis added).

Therefore, in describing how to make and use antibodies that recognize the neoepitope sequence HRGY(NO_2)PGLDG (SEQ ID NO:6), Applicant has failed to enable the skilled artisan to detect all sequence comprising that sequence, since only unwound, linear fragments present the neoepitope sequence. The antibodies taught in the specification are *not* capable of recognizing native type II collagen (as also disclosed in the instant specification), and therefore,

the specification fails to enable the skilled artisan to detect all amino acid sequences that comprise the sequence HRGYPGLDG (SEQ ID NO:6) since it fails to teach (for example) how to detect the native, full-length protein, or how to detect fragments that retain the wound triple-helix structure of collagen. In light of the postfiling literature, it is apparent that only unwound linear fragments of collagen type II that comprise the sequence would be able to be detected by the antibody of the invention.

Claims 2-4 refer to an "**immunological binding partner**" which is immunoreactive with a nitrated form of a specific protein. The claims would encompass such "immunological binding partners" that recognize nitrated residues in both a context-dependent (claim 3) and in a context-independent manner (claim 4). However, the specification is not enabling for all such "immunological binding partners" because "context-independent" antibodies would by definition recognize nitrated residues regardless of the surrounding protein sequence, such that they could not be used alone in order to detect *specific* protein sequences, since they would bind to all nitrotyrosine-containing sequences irrespective of the surrounding protein sequence. The specification only describes how to use such "context-independent" antibodies in conjunction with a second antibody (as in claim 4), and therefore does not teach the skilled artisan how to carry out the full scope of the claimed detection methods.

In addition, with respect to the claimed "immunological binding partners" recited in claims 2-4, while the specification discloses *antibodies*, no direction or guidance is provided with regard to how to make any other types of "immunological binding partners". There are no working examples in which "immunological binding partners" other than antibodies were used to

detect any amino acid sequence. The disclosure of *antibodies*, and of art-recognized methods of making *antibodies* (see pages 16-17), is not commensurate with the scope of the claims.

Turning to the use of "context-dependent" antibodies (as disclosed for example at page 5, lines 6-11 of the specification), it is noted that the specification discloses that the antisera of the invention discriminates between the nitrated and the non-nitrated form of the SEQ ID NO:6, since the antisera had only weak affinity to the non-nitrated sequence (see page 23, lines 1-6). However, the claims broadly recite only that the "immunological binding partner" is reactive with the nitrated form of the aromatic amino acid residue. Claim 3 recites that the binding partner is "specifically reactive" with the nitrated form; however, since Applicant has not defined the term "specifically reactive", the claims do not exclude antibodies that react with **both** the nitrated and the non-nitrated aromatic residue. The claims fail to clearly recite that the antibodies distinguish between the nitrated and the non-nitrated sequence. The specification fails to teach the skilled artisan how to use antibodies that bind to both nitrated and non-nitrated sequences in methods of detecting specific nitrated amino acid sequences, as currently encompassed by the claims. There are no working examples in which such antibodies were employed.

Applicant is reminded that should the claims be amended in accordance with the scope of enabling disclosure indicated above, any amendments must be supported by the original disclosure and must also comply with the requirements of 35 USC 112, 2nd paragraph.

23. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

24. Claims 1-9 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

25. Claim 1 recites an amino acid sequence which is "**characteristic of**" a specific protein, which is vague and indefinite. Applicant has not specifically defined what is meant by an amino acid sequence that is "**characteristic of**" a specific protein. It is unclear what types of amino acid sequences would be considered to be "characteristic of" proteins since no limiting definition is given. This might encompass, for example, such diverse classes of sequences as fragments, homologs, modified forms, and/or structural analogs. In the absence of a specific definition for the term in this context, the metes and bounds of the claim are unclear.

Claim Rejections - 35 USC § 102

26. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

27. Claims 1-5 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Ter Steege et al. ("Nitrotyrosine in Plasma of Celiac Disease Patients as Detected by a New Sandwich ELISA" *Free Radical Biology & Medicine* (1998) Vol. 25, pages 953-963).

Ter Steege et al. teach a method comprising detecting an amino acid sequence that is characteristic of a specific protein (IgG) and which contains one or more aromatic amino acid residues in nitrated form (see especially the abstract). In particular, Ter Steege et al. teach two

monoclonal anti-nitrotyrosine antibodies raised against nitrated KLH (see p. 955, left column, "Monoclonal antibody production"). The antibodies were used to detect nitrated IgG in an immunoassay (Western blot or ELISA) (see p. 955-956, "SDS-PAGE and Western blotting; p. 956-957, "Western blot analyses..."; Figure 2, especially lane I; p. 956, "Nitrotyrosine ELISA"; p. 957-959, "Development of a nitrotyrosine ELISA" and p. 960-961). Detection of nitrated IgG reads on detection of an amino acid sequence that is "characteristic" of this protein, since the sequence of the full-length protein would be considered to be "characteristic" of the protein.

With respect to claim 3, the immunological binding partner (monoclonal antibodies) of Ter Steege et al. is "specifically reactive" with the nitrated form of an aromatic amino acid residue (nitrotyrosine) in the context of IgG, since the antibodies bind to nitrotyrosine in the context of IgG with much higher affinity than to free nitrotyrosine (page 961, left column, the last paragraph to right column, the first full paragraph). It is noted that Applicant has not provided a specific or limiting definition for the term "specifically reactive", such that given the broadest reasonable interpretation, the claim is not limited to the use of antibodies that bind to nitrotyrosine in a strictly context-dependent manner. Thus, although the antibodies of Ter Steege et al. recognize nitrotyrosine in the context of various proteins (including nitrated BSA and nitrated KLH), the reference method nonetheless reads on the claim since the antibodies of the reference "specifically reacted" with nitrotyrosine in the context of these specific proteins.

With respect to claim 4, the antibodies of Ter Steege et al. may also be considered to be reactive with nitrotyrosine in a "context independent manner" as recited since the antibodies react with nitrotyrosine in the context of KLH, BSA, and IgG, as well as with free nitrotyrosine (see especially page 955, "Monoclonal antibody production"). The reference teaches a sandwich

ELISA in which a first anti-nitrotyrosine (context-independent) monoclonal antibody was used as a coating antibody (see p. 957-959, "Development of a nitrotyrosine ELISA"; and Figures 3 and 5). The second anti-nitrotyrosine antibody was then used as the second detection antibody (the reference teaches that either of the two anti-nitrotyrosine antibodies can be used as the coating antibody, with the other serving as detection antibody). The second detection antibody has binding specificity for IgG, since it binds to nitrated IgG, and thus may be said to have binding specificity for an amino acid sequence that is characteristic of IgG.

With respect to claim 5, *human* IgG is taught (see page 958, left column, the first full paragraph).

28. Claim 6 is rejected under 35 U.S.C. 102(b) as being anticipated by Ter Steege et al. and in light of de Vries et al. ("Specific localization of IgG isolated from inflamed synovial tissue" *Agents and Actions* Vol. 19 (1986) pages 5-6).

Ter Steege et al. is as discussed above, which teaches detection of IgG. However, the reference does not state that IgG is a protein that is present in joint tissue.

de Vries et al. is relied upon as an evidentiary reference to show that IgG is produced locally in the synovial tissue in conditions such as rheumatoid arthritis (page 5, "Introduction").

Therefore, in light of the evidence of de Vries et al., it can be seen that the teaching of IgG in Ter Steege et al. reads on the claim since IgG is a protein that is present in joint tissue.

29. Claims 1, 5, and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Mikotor et al. (WO 01/84160 A2). It is noted that the reference currently constitutes prior art under 35 USC

102(b) because Applicant has not fulfilled one or more conditions to receive benefit of the filing date of provisional application 60/363,925 (see "Priority" above). Should Applicant perfect the benefit claim, the reference may still constitute prior art under 35 USC 102(a) and/or (e).

Mikotor et al. teach a method of assaying for protein oxidative damage comprising detecting nitrotyrosine (see especially page 3, line 15 to page 6). In particular, the reference teaches detecting in a biological sample an amino acid sequence (peptide fragment) that is characteristic of a specific protein (see especially page 14, the second paragraph). The peptides are "characteristic of" specific protein since they can be used to determine the identity of the proteins of which they are fragments, by searching the protein sequence databases (ibid and page 45). By analyzing the peptide fragments by mass spectrometry, peptides that contain nitrotyrosine are detected (see also pages 24-25).

As one example, Mikotor et al. teach detection of the amino acid sequence Y(NO2)LYEIAR (see pages 38-40 and Figure 5), which is a sequence that is "characteristic of" the protein BSA since it is a predicted tryptic peptide of BSA (see page 38). The amino acid sequence contains an aromatic amino acid residue (tyrosine) in nitrated form (nitrotyrosine, Y(NO2)).

With respect to claim 5, BSA is a mammalian protein (*bovine* serum albumin).

30. Claims 1 and 5-7 rejected under 35 U.S.C. 102(b) as being anticipated by Païk et al. (*Connective Tissue Research* Vol. 42, pages 111-122 (October 2001), see Applicant's Information Disclosure Statement). This reference currently constitutes prior art under 35 USC 102(b) because Applicant has not fulfilled one or more conditions to receive benefit of the filing

date of provisional application 60/363,925 (see "Priority" above). It is noted that should Applicant perfect the benefit claim, the reference may still constitute prior art under 35 USC 102(a).

Paik et al. teach a method for detecting collagen oxidation by nitration, comprising detecting in a sample of insoluble type I collagen an amino acid sequence that is characteristic of a specific protein and which contains one or more aromatic amino acid residues (tyrosine) in nitrated form (3-nitrotyrosine) (see especially the abstract and p. 115, "Detection of 3-nitrotyrosine" and also "Non-enzymatic nitration of collagen..."; Table 1; and Figures 3-4). The reference teaches detecting proteolytic fragments of nitrated collagen type I, which may be said to be "characteristic" of collagen type I in that they originate from the full-length protein, by HPLC. See p. 115, right column, the first paragraph; Table 1, and Figure 4B. The reference also teaches detecting nitrated collagen by UV/visible absorption spectroscopy. In this case the full-length nitrated collagen is detected; such a sequence would also be said to be "characteristic" of collagen type I since a protein's sequence is one defining characteristic or property. See p. 115, "Yellow chromophore analysis"; Figure 3; Figure 5; and p. 120, left column).

Conclusion

31. Claims 1-9 are rejected.

32. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Khan et al. ("3-Nitrotyrosine in the proteins of human plasma determined by an ELISA method" *Biochem J.* (1998) 330, 795-801) is relevant to the claimed invention for its teaching of detection of nitrotyrosine-containing proteins by immunoassay.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

cfoster

Christine Foster, Ph.D.
Patent Examiner
Art Unit 1641

LONG V. LE 02/22/07
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600